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Bioactivity and properties of a dental adhesive functionalized with polyhedral oligomeric silsesquioxanes (POSS) and bioactive glass

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Keywords: dental adhesive, nanoparticles, bioactivity, viscosity, water sorption, sol fraction, conversion, network structure

Research Highlights:

- Three fillers with various functionality tailored an adhesive bioactive
- Viscosity increased due to the bioactive glass agglomerates
- Maintained or improved structural and physico-chemical properties of the adhesive through multifunctional particles

Conflict of interest:

DM declares a financial interest in the form of a patent application (WO2011/020204) on radio-opaque bioactive glass licensed to smartodont llc., of which DM is a shareholder.

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Abstract

Objectives: This study aimed to analyze the effect of infiltrating a commercial adhesive with nanosized bioactive glass (BG-Bi) particles or methacryl-functionalized polyhedral oligomeric silsesquioxanes (POSS) on material properties and bioactivity

Methods: An acetone-based dental adhesive (Solobond Plus adhesive, VOCO GmbH, Cuxhaven, Germany) was infiltrated with nanosized bioactive glass particles (0.1 or 1 wt%), or with monofunctional or multifunctional POSS particles (10 or 20wt%). Unfilled adhesive served as control. Dispersion and hydrodynamic radius of the nanoparticles were studied by dynamic light scattering. Set specimens were immersed for 28 days in artificial saliva at 37° C, and surfaces were mapped for the formation of calcium phospate (Ca/P) precipitates (scanning electron microscopy/energy-dispersive x-ray spectroscopy). Viscosity (rheometry) and the structural characteristic of the networks were studied, such as degree of conversion (FTIR spectroscopy), sol fraction and water sorption.

Results: POSS particles showed a good dispersion of the particles for both types of particles being smaller than 3 nm, while the bioactive glass particles had a strong tendency to agglomerate. All nanoparticles induced the formation of Ca/P precipitates. The viscosity of the adhesive was not or only slightly increased by POSS particle addition but strongly increased by the bioactive glass particles. The degree of conversion, water sorption and sol fraction showed a maintained or improved network structure and properties when filled with BG-Bi and multifunctional POSS, however, less polymerization was found when loading a monofunctional POSS.

Significance: Multifunctional POSS may be incorporated into dental adhesives to provide a bioactive potential without changing material properties adversely.

1. Introduction

Over the past decade, functionalization of dental adhesives to combat degradation of the dentin hybrid layer was intensively studied. The degradation of the hybrid layer is attributed to the hydrolytic and enzymatic degradation of hydrophilic resin components

and exposed collagen fibrils lacking the protection of polymerized resin [1]. Strategies to prevent the degradation of the hybrid layer and, thus, to increase the longevity of composite restorations, include the inhibition of endogenous proteases and the stimulation of dentin mineralization [1].

Nanoparticles of amorphous calcium phosphate [2,3], bioactive glass [4] or hydroxyapatite [5–7] have been incorporated into dental adhesives to induce mineral precipitation within the hybrid layer. The nanosized bioactive glass offers better antimicrobial effects and bioactivity through the higher release of alkali species than the conventional bioactive glass in microparticulate form [8]. However, fillers might increase the viscosity of the adhesive [2,4], impeding the wettability of the dentin surface and diffusion into demineralized dentin. The unbounded fillers may be washed out easily and could have potentially adverse effects.

The incorporation of the functionalized Polyhedral oligomeric silsesquioxanes (POSS) particles has been shown to improve mechanical properties, such as flexural strength, toughness and to reduce network solubility and degradation [9]. POSS are nanostructured hybrid molecules with an inorganic framework of silicon and oxygen atoms and an outer shell of organic functional groups allowing polymerization [10]. The hybrid character renders a very good dispersion and particle mobility in an organic substances different from a typical hard/compact particle [11]. The multifunctional POSS particles with highly reactive groups lead to higher cross-link density [12] and, thus, also mechanical properties may be improved. At low concentrations, POSS particles were successfully incorporated into experimental composite decreasing the volumetric shrinkage and improving the mechanical properties, e.g. flexural strength, Young`s modulus or hardness [13–16]. When applied in a dental adhesive, the fillers acting as a cross-link agent may also enhance the bond to a composite through the increase of the shear strength [17]. In the same time, POSS-containing materials were shown to promote the formation of plate-like hydroxyapatite crystals at their surfaces indicating in vitro bioactive properties [15,18–21].

Moreover, fillers like POSS particles can render an adhesive more hydrophobic and increase the monomer conversion. Residual unreacted monomers and oligomers may be extracted from the restorative material to the organism and act as toxic species [22]. When the areas released from the residuals are filled with water, the mechanical properties of the adhesive are lowered and the process of aging is accelerated [23]. Fillers might potentially reduce these adverse effects.

Therefore, the incorporation of POSS particles into dental adhesives might be of great benefit to stimulate mineralization and improve the mechanical properties of the hybrid layer. The present study aimed to analyze the effect of infiltrating a commercial adhesive with POSS compared to nanosized bioactive glass particles on bioactivity and material properties. The null hypothesis was that the studied particles will not make the adhesive bioactive and that there is no difference between the particle-filled adhesives and the neat adhesive system regarding its properties, such as viscosity, degree of conversion, water sorption and sol fraction.

2. Materials and Methods

The adhesive resin of a commercial acetone-based dental adhesive system (Solobond Plus Adhesive, VOCO GmbH, Cuxhaven, Germany) was used in this study and infiltrated with nanosized bioactive glass particles [24] or POSS (Hybrid Plastics Inc., Hattiesburg, USA). According to the manufacturer, Solobond Plus Adhesive contained acetone (> 50%), Bis-GMA (10-25%), TEGDMA (10-25%), HEMA (5-10%) and a catalyst (< 2.5%). Nanosized bioactive glass particles equivalent to Bioglass 45S5 with 20 wt% Bi_2O_3 (BG-Bi) were synthesized by flame spray synthesis as described elsewhere [24] and added at two concentrations: 0.1 or 1 wt%. Monofunctional (POSS-1) and multifunctional (POSS-8) methacryl POSS (MA 0702, Methacrylisobutyl POSS and MA 0735 - Methacryl POSS, respectively) were mixed with the adhesive in the concentrations of 10 and 20 wt% (Fig.1). The chemical structure of both POSS particles is depicted in Fig.2. The mixtures were mixed with a magnetic stirrer for 5 minutes in

dark. For the preparation of cured specimens, the solvent was subsequently removed by keeping the samples in vacuum for 10 min. Unfilled adhesive were prepared likewise and served as control. The analogous mixtures were prepared for viscosity experiments with the Solobond Plus adhesive resin without initiator (VOCO GmbH, Cuxhaven, Germany).

2.1. Dispersion and size of the particles

Acetone, as the solvent of the Solobond Plus adhesive, was used for the dynamic light scattering (DLS) experiment to study the particle size and possible tendency for aggregation. The adhesive itself is not applicable due to the contributions from various monomeric molecules (HEMA, Bis-GMA, acetone) that overlap with the typical peaks from particles. Acetone is thus a reasonable alternative, as the particles are expected to behave in a similar manner if dispersed in an adhesive with over 50% acetone (Solobond Plus adhesive) as in the pure acetone.

POSS-8 was measured at 4 concentrations: 0.5, 1, 2 and 5 wt%. Due to the additional contribution from the large particles (agglomerates or impurities), the POSS-1 and BG-Bi particles could not been measured directly. 1 wt% of particles dispersed in acetone were centrifuged for 20 min at 21000g (Fresco21, Heraus, Hanau, Germany) prior to the light scattering experiment (Zetasizer Nano-ZS, Malvern, Worcestershire, Great Britain). The supernatant was used and diluted to 5 reduced concentrations: X, X/2, X/4, X/8, X/16. The BG-Bi particles could not be analyzed due to remaining agglomerates.

DLS measures the intensity correlation function of light scattered at particles immersed in a solvent [25]. The hydrodynamic radius R of the particles is related to the measured relaxation rate Γ of the correlation function by $\Gamma = Q^2 D_0$ and the Stokes-Einstein equation

$$D_0 = k_B T / 6\pi\eta R \quad (1)$$

Here k_B is the Boltzmann constant, T represents temperature in Kelvin, η viscosity of the solvent and Q the scattering vector used for DLS. The measurements were analyzed by the instrument build in “General Purpose” mode, which relates the measured correlation function to a size distribution. Each concentration was measured 3 times for about 2 min (13x10 s) at 25 °C and the analyzed results were averaged. All measurements were made at an angle of 173°.

2.2. Bioactivity

Disk-shaped specimens (diameter: 6 mm, height: 2 mm) of each group (n = 3) were prepared in a custom-made silicon form, covered by a glass plate and light-cured for 120 s (Bluephase, Ivoclar Vivadent, Schaan, Lichtenstein, 1090 mW/cm^2). Afterwards, the specimens were removed from the mold and cured from the other side for 120 s. Specimens were stored in 15 ml artificial saliva [26] or in distilled water at 37 °C for 28 days without changing the medium.

After the storage period, all specimens were gently rinsed with water and kept in desiccators at room temperature with silica gel for slow drying. The specimens for morphological analysis were sputtered by Platinum-Palladium and inspected with scanning electron microscopy at 10kV (Ultra Plus, Carl Zeiss GmbH., Jena, Germany,). The elemental composition was determined by the energy-dispersive X-ray spectroscopy at 20kV (Cryo-FE-SEM, FEI Quanta 200 FEG with Edax, FEI Company, Hillsboro, USA).

2.3. Viscosity

The viscosity of the adhesives was measured in systems without initiators with a rheometer (ARG2, TA Instruments, New Castle, USA) using a cone-plate geometry with 60 mm diameter and 0.5° cone angle. The experiments were performed at 25 °C und in

dark. The linearity was checked by strain sweep experiment with the frequency of 10 rad/s and strain ranging from 1 to 20 % and 20 to 1 %. Afterwards, the steady rate mode from 1 rad/s to 10 rad/s was applied (n = 3). The results were fitted by Cross model, and the viscosity was taken from the region where most of the systems reached a constant value (γ = 20 1/s).

2.4. Degree of conversion

Degree of conversion was estimated using a Fourier transform infrared spectrometer (Bruker, Billerica, USA) equipped with an attenuated total reflectance device. For each measurement, 26 spectra were collected in the range 900-4000 cm^{-1} with a resolution of 4 cm^{-1}. A background measurement was performed before each experiment. A thin layer of adhesive was applied on the crystal (6 mm diameter, ~0.25 mm thickness). A time delay of 3 min was used for acetone to be evaporated [27]. After this time, the sample was cured for 20 s with a LED curing lamp (Bluephase, Ivoclar Vivadent, Schaan, Liechtenstein, 1090 mW/cm^2) and spectra were collected for the following 10 min.

The degree of conversion was estimated from the ratio of peak heights of aliphatic C=C stretching vibrations at 1638 cm^{-1} (H_{al}) and aromatic C-C stretching vibrations at 1608cm^{-1} (H_{ar}) from the background corrected spectra of cured and uncured samples:

$$DC = 1 - \frac{\left({}^{H_{al}}/_{H_{ar}}\right)_{cured}}{\left({}^{H_{al}}/_{H_{ar}}\right)_{uncured}} \quad (2)$$

The values of the cured system were taken from the plateau in the spectra at 10 min after curing.

2.5. Water sorption and sol fraction

Specimens (each n = 5), prepared in the same way as for the bioactivity test, were kept in a desiccator with silica gel to absorb humidity and weighted (Sartorius, Göttingen, Germany) each third day until the mass change Δm was below or equal 0.1 mg. The

final mass was taken as m_0. The specimens were then immersed in distilled water, kept in an incubator at 37 °C and weighted every 3 days until a stable value ($\Delta m \leq 0.1$ mg) to reach the mass m_1. The mass of the extracted dry samples m_2 was obtained after the second drying cycle in the desiccator. Each cycle lasted around 20 days.

The water sorption WS and sol fraction SF were estimated from the following equations:

$$WU = (m_1-m_2)/V_{m0} \quad (3)$$

$$SF = (m_0-m_2)/V_{m0} \quad (4)$$

where V_{m0} represents the volume of the specimen before immersion calculated from the specimen diameter and thickness measured by a micrometer. The water sorption is given by the increase in weight due to the water absorption. The sol fraction defines the fraction of the material that is not connected to the main network and is thus extracted during immersion.

2.6 Statistical Analysis

The statistical significances of the data obtained from the viscosity, degree of conversion, sol fraction and water sorption measurements were assessed using one-way ANOVA and Tukey's post hoc test ($\alpha = 0.05$). All statistical analyzes were performed using Dell Statistica, v. 12, 2015 (Dell Inc., Tulsa, USA).

3. Results

3.1. Dispersion and size of particles

A strong aggregation of BG-Bi particles in acetone made the analysis of scattering data impossible due to non-uniform sizes of the agglomerates contributing to the correlation function in the whole range of time. Agglomerates of up to the micrometer size range could be detected, however, the evaluation of the distribution of the particle radius was not possible. Both POSS particles revealed a good solubility in acetone. The diffusion coefficient obtained for several dilutions were extrapolated to $\phi \rightarrow 0$ (Fig. 3). The zero diffusion coefficient D_0 was obtained for POSS-1 (901 $\mu m^2/s$) and POSS-8 (657 $\mu m^2/s$), and the hydrodynamic radius was estimated based on the eq.1, taking the viscosity of acetone η = 0,311 mPas, as 1.1 ± 0.2 nm for POSS-1 and 0.8 ± 0.2 nm for POSS-8. These values correspond to the structural information from the producer [12] and are comparable to the dimensions of monomers used in the adhesive, although the measured values only determine a radius of ideal sphere that diffuses in the solvent with the same coefficient as the examined scatterers.

The dependence of the diffusion coefficient $D(\phi)$ on concentration ϕ is described by $D(\phi)=D_0(1+k_D\phi)$ with the interaction parameter k, which is proportional to the second virial coefficient B_2. The second virial coefficient describes interactions between molecules in a solution in a thermodynamically non-ideal state [28]. Negative values reflect predominantly attractive interactions among dispersed molecules and may lead to the precipitation or aggregation. A positive virial coefficient is indicates repulsive forces [29] which stabilize the dispersion. The obtained interaction parameters in POSS-acetone solutions indicate repulsion for POSS-1 and attraction for POSS-8 particles.

3.2. Bioactivity

The growth of the calcium phosphate precipitates on the surface was observed in all groups with the studied particles when immersed in artificial saliva for 28 days (Fig. 4).

Only the neat adhesive showed no evidence of the precipitates. Typically, the long thin plate-like crystals attached to one nucleation point appeared on the surface of specimen. The highest density of crystals was found on the adhesive filled with BG-Bi (Fig. 4a), the lowest for the POSS-1 addition (Fig. 4b). No bioactivity was observed on the specimens immersed in water.

The chemical composition of the precipitates is shown in the EDX spectra (Fig. 4). All specimen filled with particles exhibited the presence of the calcium and phosphorus peak. These were, however, absent in the unfilled adhesive and in the samples immersed in water. Crystals were found in all samples with fillers immersed in artificial saliva, however, not in the specimens immersed in water or in pure adhesive.

3.3. Viscosity

A strong shear thinning was observed in the adhesives filled with BG-Bi (data not shown). Similarly, however less intense, shear thinning appeared in the POSS-1-adhesive mixtures. For that reason, the infinite viscosity could not be determined by the Cross model fit in the range measured. The neat and the POSS-8-filled adhesive did not show distinct shear thinning.

The BG-Bi particles at 0.1 wt% loading did not significantly change the viscosity of the adhesive, however, at concentration of 1wt% the viscosity was significantly increased. No significant differences were found in the adhesives filled with POSS particles ($p > 0.05$, Tab 1). POSS-1 particles led to a systematic increase, as well as the 20 wt% POSS-8 where the viscosity was slightly higher than for the pure adhesive. Interestingly, no increase of the viscosity was obtained at 10 wt% loading of POSS-8 particles (Tab 1).

3.4. Degree of conversion

The degree of conversion was estimated according to eq.2 from the ratio of peaks for C=C stretching and C-C aromatic vibrations on Bis-GMA which served as a reference.

The unfilled and the BG-Bi particles filled adhesive exhibited a degree of conversion above 50% (Tab 1). POSS-1 particles caused a robust drop of conversion degree ($p < 0.001$). At both concentrations (10 and 20 wt%) the adhesive remained fluent even after 120 s of curing. POSS-8 reduced the degree of conversion to 43.8 ± 0.4% and 38.8 ± 0.3% at a loading of 10 and 20 wt%, respectively. The degree of conversion was significantly decreased by both POSS particles at both concentration studied ($p < 0.05$).

3.5. Water sorption and sol fraction

The results of water sorption and sol fraction experiments of pure or filled adhesive are presented in Tab 1. The hydrophilicity of the BG-Bi particles resulted in a slight but non-significant increased water sorption in comparison with the neat adhesive. On the other side, the typically hydrophobic POSS-1 particles significantly reduced the sorption almost 3-times at both studied concentrations. The sorption for POSS-8 was also decreased.

The fraction of sol of Solobond plus adhesive is comparable when filled with BG-Bi at 0.1 wt% and 1 wt% or POSS-1 at the concentration of 10 wt%. A significant reduction can be seen at 20 wt% concentration and by POSS-8.

4. Discussion

This study showed that the incorporation of the mono- and multifunctional POSS particles into the Solobond Plus adhesive renders the bioactive properties. Although the known significant bioactive properties of the BG-Bi, these particles turned out to be unsuitable as a filler for the Solobond Plus adhesive because of the agglomeration of the particles and the strong viscosity increase up to the level that the implementation in the clinical praxis would be disadvantageous. Most probably, this agglomeration could be due to the incomplete dispersion of the BG-Bi particles in the adhesive system. On the other side, in all systems filled by POSS the viscosity is still low enough for the application as adhesive.

A fair shear thinning using bioactive glass fillers was also observed when mixed with Heliobond adhesive [4] and is due to the orientation of the particles in clusters/microstructure that are shattered by the deformation at higher rate. No shear thinning was observed by POSS-8 filled adhesive because of the stable and good dispersion of the particles. This assumption is supported by the results from dynamic light scattering where no tendency for clustering for POSS-8 but a strong agglomeration of BG-Bi was observed. The increasing viscosity of the adhesives filled with BG-Bi and POSS-1 with reduced shear rate shows that the spreading of the mixture through the rough structure of a tooth will be hindered when not under stress and worse than in the case of the neat adhesive [30]. Similarly, the penetration of the systems with agglomerated particles (BG-Bi and partially POSS-1) is expected to be strongly inhibited in comparison with the adhesive filled by the well dispersed POSS-8 particles [31]. In order to optimize the dispersion of agglomerated BG-Bi particles by advanced dispersion techniques/systems further studies needs to be done.

The polymerization of monomers was not hindered by the BG-Bi particles as no reaction with the particles is expected. Other studies have shown that this kind of particle does not hinder the hardening of endodontic sealing material, too [32]. Yet, these particles tend to cluster, most of the monomers are surrounded by other monomers and have therefore a similar probability of reaction as in the unfilled adhesive. The extremely reduced degree of conversion by POSS-1 loading may be partially caused by the additional vinyl groups from POSS particles, which decrease the measured degree of conversion. Even though this effect is expected to be emphasized in POSS-8 systems with 8 vinyl groups per particle, the obtained reduction in degree of conversion by POSS-1 was much higher. Therefore, some additional mechanism - termination due to the particles, reaction with possible impurities in the POSS-1 system, interactions with initiator, steric hindrance and reduction of the mobility, etc. [33] - could play an important role in inhibition of the polymerization reaction in POSS-1 loaded adhesive.

In the adhesive mixed with POSS-8 particles the situation seems to be different. Although the lower reactivity and conversion of the POSS particles with 8 methacryl groups than those with only one group due to steric interactions was found when mixed with UDMA [34], the combination with Solobond Plus adhesive shows a different result. The measured degree of conversion of the POSS-8 loaded adhesive system is slightly lower than that of the neat adhesive but the real cross-link density and conversion of the monomers may be even increased. An increased strength of the methacryl-POSS filled composite was found even though the conversion of vinyl groups was slightly decreased [13,33]. The measured degree of conversion includes besides the C=C groups of the monomers also those from the methacryl groups on the particles. Thus, if two methacryl groups on the particle react, it may become a strong cross-link while other unreacted groups would still reduce the measured degree of conversion compared to unfilled adhesive. By considering the same conversion of monomers in filled and unfilled adhesive, the ratio of unreacted vinyl groups to benzene groups in the cured adhesive with ϕ particles would be:

$$R_f^c = R_a^c + \frac{\phi}{1-\phi}\frac{X}{B} \tag{5}$$

Where *c* refers to cured and the subscripts *f* or *a* are assigned to filled or neat adhesive, resp. The ratio of residual vinyl groups on POSS to benzene groups in the system is depicted by X/B.

The ratio of the residual vinyl groups on the POSS particles in the cured (X) versus uncured (P) filled adhesive would be

$$\frac{X}{P} = \frac{R_f^c - R_a^c}{R_f^u - R_a^u} \tag{6}$$

Here R^c specifies the vinyl-to-benzene ratio for cured system. Using these simplifications and assumptions, there would be 11% or 8% of vinyl groups on POSS-8 particles that would react additionally to the vinyl groups on the adhesive monomers at 10wt% or 20%wt loading, resp. Contrarily, the X/P >1 at both POSS-1 concentrations

(11 or 9 at 10wt% or 20wt%, resp.) manifests that the conversion of monomers in the system with POSS1 was lower than in the neat adhesive. We note, that for these assumptions a very simple model was considered where the possible different reactivity between vinyl groups of POSS particles and adhesive monomers is not considered and no differentiation between the connections of various or identical molecules can be taken into account. Nevertheless, the quantitative estimation of remaining vinyl groups serves for better comparison between the studied groups.

The results of water sorption follow the previous data on degree of conversion. The supposedly lower cross-linking of the network filled with POSS-1 particles caused a less compact network and reduced areas surrounded by a polymer network that can act as reservoir for water. Together with the hydrophobic character of these particles such adhesive systems absorb less water than the original adhesive. An opposite tendency is observed with the hydrophilic BG-Bi particles, which seem to attract water molecules also in the networks and, thus, support the water sorption. The slightly reduced sorption under the addition of POSS-8 may be due to several factors. If the cross-link density is really increased, as hypothesized above, the mesh size is reduced, together with the interconnectivity of the network pores. Such situation would naturally lead to reduced sorption [35]. Additionally, the POSS-8 particles have a slight hydrophobic character as well, which could also affect the performance of the POSS-filled adhesive system. On the other side, if the cross-link density would be too low, as by POSS-1, then again, the water could not be kept inside the specimen when no compact network is formed [36].

Although the degree of conversion of POSS-1 loaded adhesives was very low, the sol fraction was one of the lowest among all the systems. The reason arises from the final structure of POSS-1-adhesive structure. Due to the extremely low degree of conversion, a 3D network with many big open pores and dangling chains formed the specimen. In such structure, not only water molecules but also unreacted chains diffuse easily away, maybe partially already during the manipulation with the sample before immersion [36]. The monofunctionality of POSS-1 may also reduce the average

functionality of the cross-links in the network where preferentially long dangling chains or network with long mesh size are formed, leading to the lower amount of removable sol in the network. It has been shown that POSS particles with reactive groups acting as cross-linking agents can decrease the sol fraction and water sorption [9,37]. However, several methacrylate groups on POSS-8 are expected to increase the average functionality of crosslinks and form a more compact network with a lower mesh size. Considering the combination of all 3 investigated structural characteristics of the modified adhesive: degree of conversion, water sorption and sol fraction, the adhesive filled with POSS-8 particles exhibited improved properties when compared to the unfilled adhesive.

All three studied particles in combination with the adhesive showed a bioactive behavior. Even though the bioactive glass is known and popular for its bioactive properties [4,17,32–35], the bioactive characteristics of POSS particles have not been examined profoundly. The bioactivity of methacryl-functionalized POSS was proven in a single study where, mixed with polyethylene glycol, the particles induced the apatite formation on the composite surface after immersion in Dulbecco's phosphate buffered saline [21]. Since the neat POSS did not show any sign of hydroxyl apatite growth [21], the bioactivity of POSS particles seems to be conditioned by the combination with a hydrophilic network that stimulates the hydrolysis of Si-O bonds [21]. Since no bioactivity was detected on the adhesive immersed in artificial saliva, the nucleation centers in the composites cannot consist of the adhesive network. The initiation of calcium phosphate nucleation on the POSS particles is induced by the Si-OH groups on the POSS cage [15] that remain from the imperfect reaction by the production or results from the hydrolysis of Si-O and other silica ends.

It has been shown that the BG-Bi at high concentration can increase the pH up to 10.8 and also lead to HA formation in dental resin [4]. The local increase of pH due to the release of ions from bioactive glass [42,43] accelerates the bioactivity by decreasing

the HA solubility [44]. Since no ions can be released from both POSS particles the process of Ca/P precipitation and HA formation in a case of POSS particles in the adhesive is conditional upon the presence of calcium and phosphate ions. Thus, if applied in a patient, the rate of HA growth and tooth regeneration is influenced beside others by the concentration of these ions in dentinal fluid.

The structure of the Ca/P precipitates is similar in all studied groups. These morphologies differ among studies on bioactivity [21,41] and are affected by several factors – composition and pH of artificial saliva or simulated body fluid, density of nucleation points, dynamic of calcium and phosphate ions over time, hydrophilicity, etc [45,46]. Higher concentration of bioactive particles results in more nucleation points on the surface, if dispersed properly, which initiates the formation of crystals simultaneously on more places over the specimen [4]. The growth rate as well as the critical radius for nucleation drop rapidly with the reduction of calcium and phosphate ions in the solution [44]. Due to the relatively high amount of these ions in the artificial saliva used in this study, several layers of plate-like crystals could grow over each nucleation point and expand up to 20 μm (Fig. 4). This structure is particularly interesting for dental medicine because of the plate-like morphology of enamel [18].

5. **Conclusion**

In conclusion, our study showed that POSS particles have a similar bioactive potential to bioactive glass when mixed with the adhesive while keeping the mechanical properties of the system favorable. The effect of the multifunctional POSS nanoparticles on the degree of conversion, sol fraction and water sorption indicate an improved cross-link density of the filled adhesive while keeping the viscosity low. Multifunctional POSS is therefore an interesting candidate to be tested in bond strength experiments, as it might increase bond strength due to its improved cross-linking ability and prevent bond strength degradation over time due to its mineralizing capacity.

As monofunctional POSS particles led to a strongly decreased conversion and bioactive glass particles induced a strong enhancement of viscosity, their use in dental adhesives seems to be restricted to certain applications and materials.

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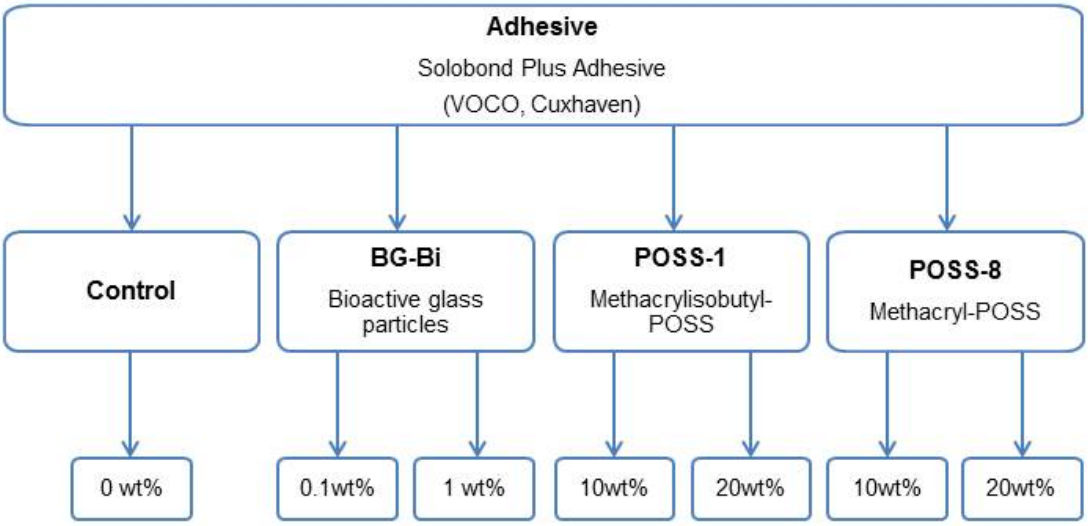

Fig. 1 The groups distribution in the present study.

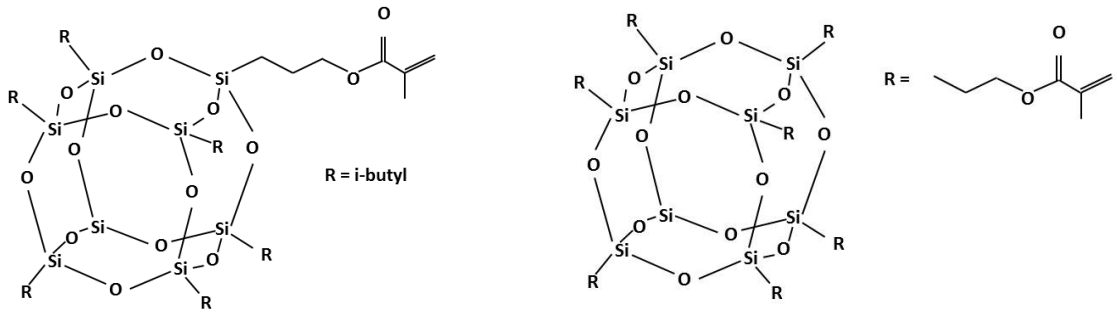

Fig. 2 Depiction of POSS-1 (left) and POSS-8 (right) particles.

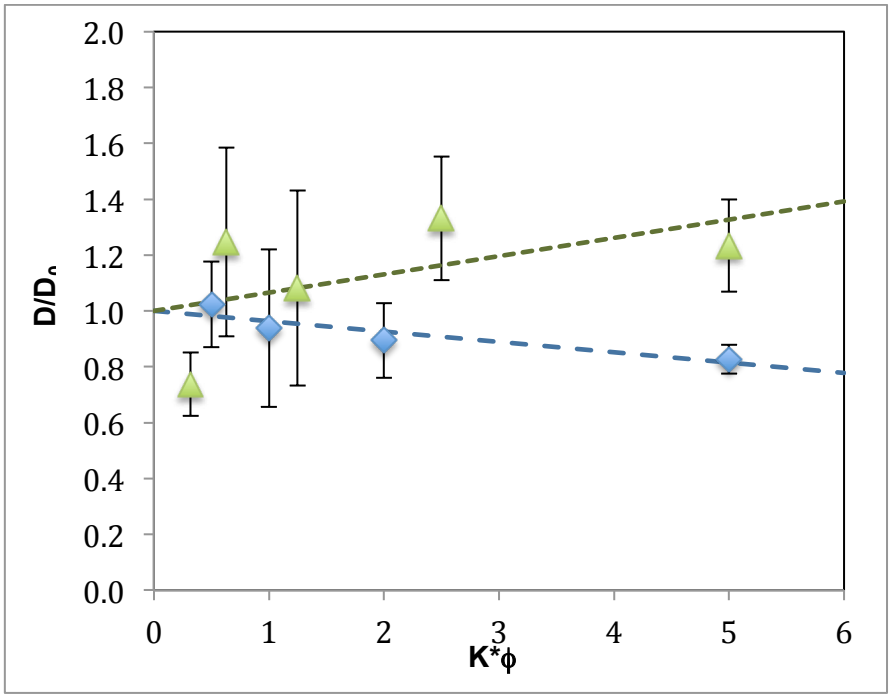

Fig. 3 Normalized diffusion coefficient of POSS-8 (blue diamonds) and POSS-1 (green triangles) as a function of volume fraction ϕ multiplied by a constant K. The linear extrapolation is shown as dashed (POSS-8) and dotted (POSS-1) line, respectively. The slope λ (POSS-8) = -0.037 and λ (POSS-1) = 0.065 yields the interaction parameter.

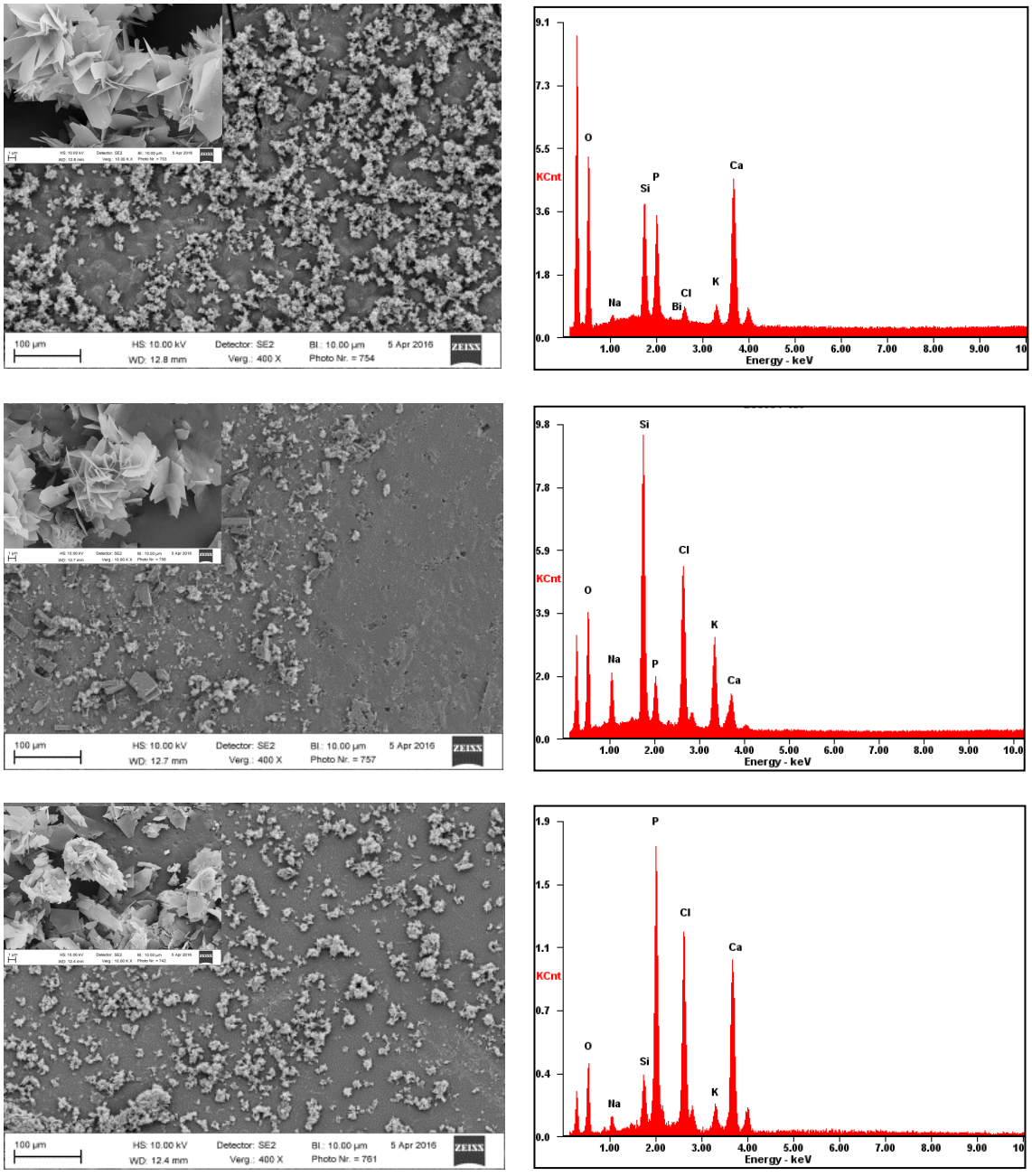

Fig. 4 a-c Representative SEM images (left) and EDX spectra (right) on adhesive filled with particles after 28 days of immersion in SBF (from up 1 wt% BG-Bi, 10 wt% POSS-1 and 10 wt% POSS-8).

Table 1 – Mean values and standard deviations of viscosity (η), degree of conversion (DC), water sorption (WS) and sol fraction (SF) of the studied systems. Different superscripts present significant differences between the groups in each column.

Fillers	Concentration [wt%]	η [mPas]	DC [%]	WS [μg/mm^3]	SF [μg/mm^3]
Control	0	21 ± 4^{a}	53.0 ± 0.7^{a}	145 ± 10^{ac}	110 ± 12^{a}
BG-Bi	0.1	23 ± 1^{a}	51.6 ± 0.5^{a}	163 ± 30^{a}	94 ± 18^{ab}
	1	334 ± 59^{b}	52.0 ± 0.7^{a}	151 ± 40^{ac}	95 ± 9^{ab}
POSS-1	10	42 ± 8^{a}	10.3 ± 1.9^{b}	52 ± 4^{bd}	88 ± 29^{ab}
	20	82 ± 2^{a}	9.3 ± 0.8^{b}	48 ± 3^{b}	56 ± 11^{bc}
POSS-8	10	19 ± 1^{a}	43.8 ± 0.4^{c}	117 ± 22^{ce}	75 ± 8^{bc}
	20	40 ± 7^{a}	38.8 ± 0.3^{d}	96 ± 20de	58 ± 6^{c}